probability. The enhanced asynchronous release in complexin-deficient mice slowed-down the recovery of synchronous EPSCs after stimulus trains suggesting both, synchronous and asynchronous release events, were fed by a common pool of vesicles.

Emerging Techniques

2556-Pos Board B575
Bringing Deconvolution Algorithmic Techniques to the Electron Microscope
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The Scanning Electron Microscope (SEM) as a three dimensional imaging tool is increasingly popular for studies in neurobiology. Serial slicing methods based on diamond-knife cutting are, however, reaching practical limitations in terms of achievable z-resolution and voxel isotropy. While Focused-Ion Beam SEM serial block face imaging can improve the z-resolution to 5nm, this technology is restricted in terms of the total volume of material that can be processed. In this work we describe the Multi Energy Confocal Scanning Electron Microscope or ''MECSEM'' method that achieves high isotropic resolution by a combination of backscatter image sequence acquisition and deconvolution (DC).
The method is built on improved understanding of beam-sample interaction for classically prepared samples. Furthermore the point spread function (PSF) of backscatter electrons (BSE) in these materials appears to be well confined laterally. As the range of penetration in the sample is dependent on the energy of the primary beam, acquiring an image sequence with increasing landing energies leads to the acquisition of images from increasing depth.
The lateral confinement of BSE PSFs allows for restricting the DC to a layer separation task (z direction). As the structure of the PSF is difficult to obtain experimentally it will be considered unknown leading to a blind DC problem requiring the recovery of both depth layers and PSFs.
To verify the reconstruction results we combine this technique with classical FIB-SEM serial block imaging using the through the lens detector in BSE mode on an FEI Helios 650 DualBeam. The z-resolution was controlled by varying the primary beam energy. The comparison with finer FIB reconstruction shows identical structures on the studied samples proving the reliability of the 3D technique, while the achieved depth using DC can reach up to 180nm.

2557-Pos Board B576
A Likelihood based Approach for Building Trajectories from Intermittent Observations
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Single Particle Tracking (SPT) using probes that blink, bleach, activate, bind or are otherwise not consistently observed throughout an experiment presents a challenge when connecting the set of observed positions into trajectories. One method for approaching the connection problem is to assign each possible connection, fluorophore 'birth' and 'death' a cost and arrange these costs into a 'cost matrix [1].' Connections, as well as 'birth' and 'death' events are found by minimizing the total cost. We show that when the costs are calculated using the known kinetic behavior of the probes and a known diffusion constant this approach can be used to find the set of connections, etc. that approximates the global maximum likelihood solution.
Although this method can find the approximate maximum likelihood solution, in many cases a favored trajectory assignment has a likelihood that is not significantly larger than a conflicting trajectory assignment. This ambiguity arises when the probe density increases such that the likelihood cannot reliably distinguish between true and false connections. using an iterative method to relax the costs of a chosen set of connections, we have been able to dramatically reduce the number of false connections with the trade off being trajectories broken at places of ambiguity and therefore more, but shorter trajectories. In order to demonstrate the effectiveness of removing such ambiguities, we show results from simulations as well as experimental data from tracking membrane proteins labeled with quantum dots or a fluorogen activating peptide system.
[1] Jaqaman , K. et al. Nature Methods v.5 no.8, (August 2008): 695-702

2558-Pos Board B577
Single-Image Molecular Analysis for Accelerated Fluorescence Imaging
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We have developed a new single-molecule fluorescence imaging analysis method, SIMA, that improves the temporal resolution of single-molecule localization and tracking studies to millisecond timescales without compromising the nanometer range spatial resolution [1,2,3,4]. In this method, the width of the fluorescence intensity profile of a static or a mobile molecule, imaged using submillisecond to milliseconds exposure time, is used for localization and dynamics analyses. We apply this method to three single-molecule investigations: (1) axial localization precision measurements, (2) subdiffraction molecular separation measurements, and (3) protein diffusion coefficient measurements in free solution. Applications of SIMA in studying intraflagellar transport processes and photosynthetic antenna complex energy transfer mechanisms will also be discussed.
[1] Shawn DeCenzo, Michael C. DeSantis, and Y. M. Wang, ''Single-image separation measurements of two unresolved fluorophores,'' Optics Express, 18, 16628-16639, (2010)
[2] M. DeSantis, S. DeCenzo, J. L. Li, and Y. M. Wang, ''Precision analysis for standard deviation measurements of single fluorescent molecule images,'' Optics Express, 18, 6563-6576, (2010)
[3] Shannon Zareh, Michael C. DeSantis, J. Kessler, J. L. Li, and Y. M. Wang, ''Single-image diffusion coefficient measurement of proteins in free solution,'' Biophysical Journal, 102, 1685-1691, (2012)
[4] M. DeSantis, S. Zareh, X. L. Li, R. Blankenship, and Y. M. Wang, ''Single-image axial localization precision analysis for individual fluorophores,'' Optics Express, 20, 3057-3065, (2012)

2559-Pos Board B578
Selective Oligonucleotide and MRNA Pull-Down with Shielded Covalent Probes
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Shielded covalent (SC) probes combine programmable base pairing, molecular conformation change, and activatable covalent crosslinking to achieve selective and durable capture of nucleic acid targets, including efficient discrimination of SNPs. Capture yields appear consistent with the thermodynamics of probe/target hybridization, allowing rational probe design. We will demonstrate RNA pull-down using surface-immobilized SC probes, exploiting covalent target capture to remove unwanted material using stringent washes, and then reversing the crosslinks to recover the targets. RNA pull-downs using SC probes will provide a powerful framework for exploring the in vivo binding partners of RNAs.

2560-Pos Board B579
Thioamides: Minimalist Chromophores for Monitoring Protein Conformation
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We have recently shown that the thioamide - a single-atom substitution of the peptide backbone - can be used as a probe to monitor structural changes in proteins by quenching fluorophores, including the natural amino acids tryptophan and tyrosine, and several unnatural amino acids. We have developed methods for incorporating the thioamides into full-sized proteins by chemically synthesizing peptides containing the thioamides, and ligating them to proteins expressed in E. coli cells. Donor fluorophores can be incorporated into the cellularly-expressed fragment using unnatural amino acid mutagenesis or post-expression labeling, so that double-labeled proteins can be generated with a minimum of unnecessary peptide synthesis. Development of these methods allows us to begin study of the role of protein motion in processes such as cell signaling and amyloid diseases.

2561-Pos Board B580
Studying T-Cell Co-Receptors with Magnetic Probes
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The interaction between T-Cells and Antigen Presenting Cells(APC) is a key process in the adaptive immune system. Different proteins are organized into a so-called immunological synapse (IS) at the interface between T-Cell and APC. The IS plays a crucial role in T-cell activation. While a lot is known about the proteins involved and their organization within the IS some aspects are still not well understood, such as the formation of the T-Cell Receptor (TCR) and associated co-receptors into microclusters that merge to form the IS. We have developed a new biophysical approach that allows us to study receptor clustering in relation to recruitment of associated proteins in the IS.